SEQUENCE LISTING

<110> DeAngelis, Paul

<120> HEPARIN/HEPAROSAN SYNTHASE FROM P. MULTOCIDA, SOLUBLE AND SINGLE ACTION CATALYSTS THEREOF AND METHODS OF MAKING AND USING SAME

- <130> 4599.014
- <150> 60/458,939
- <151> 2003-03-31
- <150> 10/142,143
- <151> 2002-05-08
- <150> 60/289,554
- <151> 2001-05-08
- <150> 60/296,386
- <151> 2001-06-06
- <150> 60/303,691
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- <150> 60/313,258
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<213> Pasteurella multocida

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Thr Tyr Phe Ala Lys Asn Thr Gly Ile Leu Lys Ser Lys Gly Asp Ile 165 170 175

Ile Phe Phe Gln Asp Ser Asp Asp Val Cys His His Glu Arg Ile Glu Page 3

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Page 4

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440

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Arg Val Asn Lys Tyr Phe Ser Ser Asp Arg Ile Val Tyr Asn Phe Gln 485 490 495

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Glu His Pro Gly Met Val Asp Ile Tyr Phe Ser Ile Leu Cys Lys 530 540

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Glu Asp Asn Lys Asn Thr Glu Thr Leu Phe His Glu Phe Gln Asn Arg 565 570 575

Asp Glu Ile Gln Ser Lys Leu Ile Ile Ser Asn Asn Pro Trp Gly Tyr 580 585 590

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<212> DNA

<213> Pasteurella multocida

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Page 8

Lys Pro Leu Glu Asn Asp Thr Ala Val Asn Ile Leu Gly Thr Gly Thr 500 505 510

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Asn Asn Ile Leu Gln Val Cys Ile Ser Arg Pro Ser Asn Trp Leu Thr 545 550 555 560

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<213> Pasteurella multocida

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<212> PRT

<213> Pasteurella multocida

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Lys Val Asn Asn Met Asp Tyr Arg Leu Gly Phe Île Thr Leu Gly Met 260 265 270

His Lys Lys Val Phe Gln Glu Île Gly Phe Phe Asn Cys Thr Thr Lys

Page 11

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Page 12

Gly Thr Val Ser Phe Arg Val Ser Leu Phe Asn Gln Phe Ser Leu Ser 545 550 555 560

Asp Phe Thr His Ser Gly Met Ala Asp Ile Tyr Phe Ser Leu Leu Cys 565 570 575

Lys Lys Asn Asn Ile Leu Gln Ile Cys Ile Ser Arg Pro Ala Asn Trp 580 585 590

Leu Thr Glu Asp Asn Arg Asp Ser Glu Thr Leu Tyr His Gln Tyr Arg 595 600 605

Asp Asn Asp Glu Gln Gln Thr Gln Leu Ile Met Glu Asn Gly Pro Trp 610 615 620

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<212> PRT

<213> Escherichia coli

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Cys Leu Asn Glu Phe Glu Glu Ile Pro Glu Glu Leu Asp Gly Phe Ser 35 40 45

Lys Leu Asn Pro Val Ile Pro Asp Lys Asp Tyr Lys Asp Val Gly Lys 50 55 60

Phe Ile Phe Pro Cys Ala Lys Asn Asp Met Ile Val Leu Thr Asp Asp 65 70 75 80

Asp Ile Ile Tyr Pro Pro Asp Tyr Val Glu Lys Met Leu Asn Phe Tyr Page 13

95

Asn Ser Phe Ala Ile Phe Asn Cys Ile Val Gly Ile His Gly Cys Ile $100 \hspace{1cm} 105 \hspace{1cm} 110$

Tyr Ile Asp Ala Phe Asp Gly Asp Gln Ser Lys Arg Lys Val Phe Ser 115 120 125

Phe Thr Gln Gly Leu Leu Arg Pro Arg Val Val Asn Gln Leu Gly Thr 130 140

Gly Thr Val Phe Leu Lys Ala Asp Gln Leu Pro Ser Leu Lys Tyr Met 145 150 155 160

Asp Gly Ser Gln Arg Phe Val Asp Val Arg Phe Ser Arg Tyr Met Leu 165 170 175

Glu Asn Glu Ile Gly Met Ile Cys Val Pro Arg Glu Lys Asn Trp Leu 180 185 190

Arg Glu Val Ser Ser Gly Ser Met Glu Gly Leu Trp Asn Thr Phe Thr 195 200 205

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<211> 520

<212> PRT

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Ile His Lys Arg Ile Asn Glu Tyr Asp Asn Val Leu Glu Leu Ser Lys Page 14

Asn Val Ser Ala Gln Asn Ser Gly Asn Glu Phe Ser Tyr Leu Leu Gly 80

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Gln Thr Tyr Glu Asn Ile Glu Ile Leu Val Cys Asp Asp Cys Ser Ser 290 295 300

Asp Lys Ser Leu Glu Ile Ile Lys Ser Ile Ala Tyr Ser Ser Arg Page 15

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Asn Glu Leu Ile Lys Lys Ala His Gly Asn Phe Ile Thr Phe Gln Asp 340 345 350

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Tyr Thr Lys Ser Asn Glu Ile Ala Leu Leu Glu Lys Tyr Thr Thr Leu 210 215 220

Phe Ser Leu Ser Ala Asn Glu Thr Glu Ile Thr Phe Asn Asp Thr Lys 235 230 240

Val Ser Glu Tyr Leu Val Ser Glu Leu Ile Lys Glu Arg Thr Glu Val Page 17

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Page 18

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Lys Ser Lys Gly Asp Ile Ile Phe Phe Gln Asp Ser Asp Asp Val Cys 85 90 95

His His Glu Arg Ile Glu Arg Cys Val Asn Ala Leu Leu Ser Asn Lys 100 105 110

Asp Asn Ile Ala Val Arg Cys Ala Tyr Ser Arg Ile Asn Leu Glu Thr 115 120 125

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Val Ile Val Val Asp Asp Tyr Ser Thr Asp Lys Thr Phe Gln Ile Ala 50 60

Ser Arg Ile Ala Asn Ser Thr Ser Lys Val Lys Thr Phe Arg Leu Asn 65 70 75 80

Ser Asn Leu Gly Thr Tyr Phe Ala Lys Asn Thr Gly Ile Leu Lys Ser 85 90 95

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' <212> PRT

<213> Pasteurella multocida

<400> 25

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Glu Ser Leu Val Lys Tyr Asn Ile Asp Ile Cys Lys Lys Asn Ile Thr 35 40 45

Gln Ser Lys Ser Asn Lys Ile Glu Glu Asp Asn Ile Ser Gly Glu Asn 50 55 60

Lys Phe Ser Val Ser Ile Lys Asp Leu Tyr Asn Glu Ile Ser Asn Ser 65 70 75 80

Glu Leu Gly Ile Thr Lys Glu Arg Leu Gly Ala Pro Pro Leu Val Ser Ile Ile Met Thr Ser His Asn Thr Glu Lys Phe Ile Glu Ala Ser Ile Asn Ser Leu Leu Gln Thr Tyr Asn Asn Leu Glu Val Ile Val Val 115 120 125 Asp Asp Tyr Ser Thr Asp Lys Thr Phe Gln Ile Ala Ser Arg Ile Ala 130 140 Asn Ser Thr Ser Lys Val Lys Thr Phe Arg Leu Asn Ser Asn Leu Gly Thr Tyr Phe Ala Lys Asn Thr Gly Ile Leu Lys Ser Lys Gly Asp Ile 165 170 175 Ile Phe Phe Gln Asn Ser Asn Asp Val Cys His His Glu Arg Ile Glu
180 185 190 Arg Cys Val Asn Ala Leu Leu Ser Asn Lys Asp Asn Ile Ala Val Arg 200 Cys Ala Tyr Ser Arg Ile Asn Leu Glu Thr Gln Asn Ile Ile Lys Val 210 215 220 Asn Asp Asn Lys Tyr Lys Leu Gly Leu Ile Thr Leu Gly Val Tyr Arg 225 230 235 240 Lys Val Phe Asn Glu Ile Gly Phe Phe Asn Cys Thr Thr Lys Ala Ser 245 250 255 Asp Asp Glu Phe Tyr His Arg Ile Ile Lys Tyr Tyr Gly Lys Asn Arg 260 265 270 Ile Asn Asn Leu Phe Leu Pro Leu Tyr Tyr Asn Thr Met Arg Glu Asp 275 280 285 Ser Leu Phe Ser Asp Met Val Glu Trp Val Asp Glu Asn Asn Ile Lys 290 Gln Lys Thr Ser Asp Ala Arg Gln Asn Tyr Leu His Glu Phe Gln Lys 305 310 315 320 Ile His Asn Glu Arg Lys Leu Asn Glu Leu Lys Glu Ile Phe Ser Phe 325 330 335

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Pro Arg Ile His Asp Ala Leu Pro Ile Ser Lys Glu Met Ser Lys Leu 340 345 350 Ser Asn Pro Lys Ile Pro Val Tyr Ile Asn Ile Cys Ser Ile Pro Ser 355 360 365 Ile Lys Gln Leu Gln Tyr Thr Ile Gly Val Leu Lys Asn Gln Cys 370 380 Asp His Phe His Ile Tyr Leu Asp Gly Tyr Pro Glu Val Pro Asp Phe 385 390 395 400 Ile Lys Lys Leu Gly Asn Lys Ala Thr Val Ile Asn Cys Gln Asn Lys 405 410 415 Asn Glu Ser Ile Arg Asp Asn Gly Lys Phe Ile Leu Leu Glu Lys Leu 420 425 430 Ile Lys Glu Asn Lys Asp Gly Tyr Tyr Ile Thr Cys Asp Asp Asp Ile 435 440 445 Arg Tyr Pro Ala Asp Tyr Ile Asn Thr Met Ile Lys Lys Ile Asn Lys 450 455 460 Tyr Asn Asp Lys Ala Ala Ile Gly Leu His Gly Val Ile Phe Pro Ser 465 470 475 480 Arg Val Asn Lys Tyr Phe Ser Ser Asp Arg Ile Val Tyr Asn Phe Gln
485 490 495 Lys Pro Leu Glu Asn Asp Thr Ala Val Asn Ile Leu Gly Thr Gly Thr 500 505 510 Val Ala Phe Arg Val Ser Ile Phe Asn Lys Phe Ser Leu Ser Asp Phe 515 520 525 Glu His Pro Gly Met Val Asp Ile Tyr Phe Ser Ile Leu Cys Lys 530 540 Asn Asn Ile Leu Gln Val Cys Ile Ser Arg Pro Ser Asn Trp Leu Thr 545 550 555 560 560 Glu Asp Asn Lys Asn Thr Glu Thr Leu Phe His Glu Phe Gln Asn Arg Asp Glu Ile Gln Ser Lys Leu Ile Ile Ser Asn Asn Pro Trp Gly Tyr

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<211> 1854

<212> DNA

<213> Pasteurella multocida

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agagtcaaca	agtattttc	atcagacaga	attgtctata	attttcaaaa	acctttagaa	1500
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<211> 617

<212> PRT

<213> Pasteurella multocida

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Glu Ser Leu Val Lys Tyr Asn Ile Asp Ile Cys Lys Lys Asn Ile Thr 35 40 45

Gln Ser Lys Ser Asn Lys Ile Glu Glu Asp Asn Ile Ser Gly Glu Asn 50 55 60

Lys Phe Ser Val Ser Ile Lys Asp Leu Tyr Asn Glu Ile Ser Asn Ser 65 70 75 80

Glu Leu Gly Ile Thr Lys Ġlu Arg Leu Gly Ala Pro Pro Leu Val Ser 85 90 95

Ile Ile Met Thr Ser His Asn Thr Glu Lys Phe Ile Glu Ala Ser Ile $100 \hspace{1cm} 105 \hspace{1cm} 110$

Asn Ser Leu Leu Gln Thr Tyr Asn Asn Leu Glu Val Ile Val Val 115 120 125

Asp Asp Tyr Ser Thr Asp Lys Thr Phe Gln Ile Ala Ser Arg Ile Ala Page 40 Asn Ser Thr Ser Lys Val Lys Thr Phe Arg Leu Asn Ser Asn Leu Gly 145 150 160

135

Thr Tyr Phe Ala Lys Asn Thr Gly Ile Leu Lys Ser Lys Gly Asp Ile 165 170 175

Ile Phe Phe Gln Asp Ser Asp Asp Val Cys His His Glu Arg Ile Glu 180 185 190

Arg Cys Val Asn Ala Leu Leu Ser Asn Lys Asp Asn Ile Ala Val Arg 195 200 205

Cys Ala Tyr Ser Arg Ile Asn Leu Glu Thr Gln Asn Ile Ile Lys Val 210 215 220

Asn Asp Asn Lys Tyr Lys Leu Gly Leu Ile Thr Leu Gly Val Tyr Arg 225 230 235 240

Lys Val Phe Asn Glu Ile Gly Phe Phe Asn Cys Thr Thr Lys Ala Ser 245 250 255

Asp Asp Glu Phe Tyr His Arg Ile Ile Lys Tyr Tyr Gly Lys Asn Arg 260 265 270

Ile Asn Asn Leu Phe Leu Pro Leu Tyr Tyr Asn Thr Met Arg Glu Asp 275 280 285

Ser Leu Phe Ser Asp Met Val Glu Trp Val Asp Glu Asn Asn Ile Lys 290 295 300

Gln Lys Thr Ser Asp Ala Arg Gln Asn Tyr Leu His Glu Phe Gln Lys 305 310 315 320

Ile His Asn Glu Arg Lys Leu Asn Glu Leu Lys Glu Ile Phe Ser Phe 325 330 335

Pro Arg Ile His Asp Ala Leu Pro Ile Ser Lys Glu Met Ser Lys Leu 340 345 350

Ser Asn Pro Lys Ile Pro Val Tyr Ile Asn Ile Cys Ser Ile Pro Ser 355 360 365

Arg Ile Lys Gln Leu Gln Tyr Thr Ile Gly Val Leu Lys Asn Gln Cys 370 380

Asp His Phe His Ile Tyr Leu Asp Gly Tyr Pro Glu Val Pro Asp Phe Page 41 390

Ile Lys Lys Leu Gly Asn Lys Ala Thr Val Ile Asn Cys Gln Asn Lys 405 410 415

Asn Glu Ser Ile Arg Asp Asn Gly Lys Phe Ile Leu Leu Glu Lys Leu 420 425 430

Ile Lys Glu Asn Lys Asp Gly Tyr Tyr Ile Thr Cys Asn Asp Asn Ile 435 440 445

Arg Tyr Pro Ala Asp Tyr Ile Asn Thr Met Ile Lys Lys Ile Asn Lys 450 455 460

Tyr Asn Asp Lys Ala Ala Ile Gly Leu His Gly Val Ile Phe Pro Ser 465 470 475 480

Arg Val Asn Lys Tyr Phe Ser Ser Asp Arg Ile Val Tyr Asn Phe Gln 485 490 495

Lys Pro Leu Glu Asn Asp Thr Ala Val Asn Ile Leu Gly Thr Gly Thr 500 505 510

Val Ala Phe Arg Val Ser Ile Phe Asn Lys Phe Ser Leu Ser Asp Phe 515 520 525

Glu His Pro Gly Met Val Asp Ile Tyr Phe Ser Ile Leu Cys Lys Lys 530 540

Asn Asn Ile Leu Gln Val Cys Ile Ser Arg Pro Ser Asn Trp Leu Thr 545 550 555 560

Glu Asp Asn Lys Asn Thr Glu Thr Leu Phe His Glu Phe Gln Asn Arg 565 570 575

Asp Glu Ile Gln Ser Lys Leu Ile Ile Ser Asn Asn Pro Trp Gly Tyr 580 585 590

Ser Ser Ile Tyr Pro Leu Leu Asn Asn Asn Ala Asn Tyr Ser Glu Leu 595 600 605

Ile Pro Cys Leu Ser Phe Tyr Asn Glu 610 615

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31

<210> 32

<211> 21

<212> PRT

<213> artificial sequence

<220>

<223> synthetic peptide

<400> 32

Lys Gly Asp Ile Ile Phe Phe Gln Asp Ser Asp Asp Val Cys His His 1 10 15

Glu Arg Ile Glu Arg 20

<210> 33

<211> 1956

<212> DNA

<213> Pasteurella multocida

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<213> Pasteurella multocida

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Asp Ser Leu Lys Thr Thr Leu Asn Lys Asp Ile Ile Ser Gln Gln Thr 35 40 45

Leu Ala Lys Gln Asp Ser Lys His Pro Leu Ser Ala Ser Leu Glu 50 60 Asn Glu Asn Lys Leu Leu Leu Lys Gln Leu Gln Leu Gln Glu 65 70 75 80 Phe Glu Lys Ile Tyr Thr Tyr Asn Gln Ala Leu Glu Ala Lys Leu Glu 85 90 95 Lys Asp Lys Gln Thr Thr Ser Ile Thr Asp Leu Tyr Asn Glu Val Ala 100 105 110 Lys Ser Asp Leu Gly Leu Val Lys Glu Thr Asn Ser Ala Asn Pro Leu 115 120 125 Val Ser Ile Ile Met Thr Ser His Asn Thr Ala Gln Phe Ile Glu Ala 130 135 140 Ser Ile Asn Ser Leu Leu Leu Gln Thr Tyr Lys Asn Ile Glu Ile Ile 145 150 155 160 Ile Val Asp Asp Ser Ser Asp Asn Thr Phe Glu Ile Ala Ser Arg 165 170 175Ile Ala Asn Thr Thr Ser Lys Val Arg Val Phe Arg Leu Asn Ser Asn 180 185 190 Leu Gly Thr Tyr Phe Ala Lys Asn Thr Gly Ile Leu Lys Ser Lys Gly 195 200 205 Asp Ile Ile Phe Phe Gln Asp Ser Asp Asp Val Cys His His Glu Arg 210 215 220 Ile Glu Arg Cys Val Asn Ile Leu Leu Ala Asn Lys Glu Thr Ile Ala 225 230 235 240 Val Arg Cys Ala Tyr Ser Arg Leu Ala Pro Glu Thr Gln His Ile Ile 245 250 255 Lys Val Asn Asn Met Asp Tyr Arg Leu Gly Phe Ile Thr Leu Gly Met 260 265 270 His Arg Lys Val Phe Gln Glu Ile Gly Phe Phe Asn Cys Thr Thr Lys 275 280 285 Gly Ser Asp Asp Glu Phe Phe His Arg Ile Ala Lys Tyr Tyr Gly Lys 290 295 300

Glu Lys Ile Lys Asn Leu Leu Leu Pro Leu Tyr Tyr Asn Thr Met Arg 305 310 315 Glu Asn Ser Leu Phe Thr Asp Met Val Glu Trp Ile Asp Asn His Asn Ile Ile Gln Lys Met Ser Asp Thr Arg Gln His Tyr Ala Thr Leu Phe 340 345 350 Gln Ala Met His Asn Glu Thr Ala Ser His Asp Phe Lys Asn Leu Phe Gln Phe Pro Arg Ile Tyr Asp Ala Leu Pro Val Pro Gln Glu Met Ser 370 375 380 Lys Leu Ser Asn Pro Lys Ile Pro Val Tyr Ile Asn Ile Cys Ser Ile 385 390 395 400 Pro Ser Arg Ile Ala Gln Leu Arg Arg Ile Ile Gly Ile Leu Lys Asn 405 410 415 Gln Cys Asp His Phe His Ile Tyr Leu Asp Gly Tyr Val Glu Ile Pro 420 425 430 Asp Phe Ile Lys Asn Leu Gly Asn Lys Ala Thr Val Val His Cys Lys 435 440 445 Asp Lys Asp Asn Ser Ile Arg Asp Asn Gly Lys Phe Ile Leu Leu Glu 450 455 460 Glu Leu Ile Glu Lys Asn Gln Asp Gly Tyr Tyr Ile Thr Cys Asp Asp 465 470 475 Asp Ile Ile Tyr Pro Ser Asp Tyr Ile Asn Thr Met Ile Lys Lys Leu 485 490 495 Asn Glu Tyr Asp Asp Lys Ala Val Ile Gly Leu His Gly Ile Leu Phe 500 510 Pro Ser Arg Met Thr Lys Tyr Phe Ser Ala Asp Arg Leu Val Tyr Ser 515 520 525 Phe Tyr Lys Pro Leu Glu Lys Asp Lys Ala Val Asn Val Leu Gly Thr 530 540 Gly Thr Val Ser Phe Arg Val Ser Leu Phe Asn Gln Phe Ser Leu Ser 545 550 560 Asp Phe Thr His Ser Gly Met Ala Asp Ile Tyr Phe Ser Leu Leu Cys 565 570 575

Lys Lys Asn Asn Ile Leu Gln Ile Cys Ile Ser Arg Pro Ala Asn Trp 580 585 590

Leu Thr Glu Asp Asn Arg Asp Ser Glu Thr Leu Tyr His Gln Tyr Arg 595 600 605

Asp Asn Asp Glu Gln Gln Thr Gln Leu Ile Met Glu Asn Gly Pro Trp 610 620

Gly Tyr Ser Ser Ile Tyr Pro Leu Val Lys Asn His Pro Lys Phe Thr 625 630 635 640

Asp Leu Ile Pro Cys Leu Pro Phe Tyr Phe Leu 645 650